Developmental Biology 328 (2009) 118–126

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Jagged 1 is necessary for normal mouse lens formation

Tien T. Le, Kevin W. Conley, Nadean L. Brown *

Division of Developmental Biology, Children's Hospital Research Foundation, Departments of Pediatrics and Ophthalmology, University of Cincinnati College of Medicine, 3333 Burnet Avenue, Cincinnati, OH, 45229, USA

ARTICLE INFO

Article history:
Received for publication 15 October 2008
Revised 6 January 2009
Accepted 9 January 2009
Available online 20 January 2009

Keywords:
Lens development
Lens fiber differentiation
Aphakia
Notch signaling
Jagged1
Proliferation

ABSTRACT

In mammals, two spatially and temporally distinct waves of fiber cell differentiation are crucial steps for normal lens development. In between these phases, an anterior growth zone forms in which progenitor cells migrate circumferentially, terminally exit the cell cycle and initiate differentiation at the lens equator. Much remains unknown about the molecular pathways orchestrating these processes. Previously, the *Notch* signal transduction pathway was shown to be critical for anterior lens progenitor cell growth and differentiation. However, the ligand or ligand(s) that direct these events are unknown. Using conditional gene targeting, we show that *Jagged1* is required for lens fiber cell genesis, particularly that of secondary fiber cells. In the absence of *Jagged1*, the anterior growth and equatorial transition zones fail to develop fully, with only a handful of differentiated fiber cells present at birth. Adult *Jagged1* conditional mutants completely lack lenses, along with severe anterior chamber deformities. Our data support the hypothesis that *Jagged1-Notch* signaling conveys a lateral inductive signal, which is indispensable for lens progenitor cell proliferation and differentiation.

Introduction

A hierarchy of genes regulate tissue morphogenesis, growth and two waves of fiber cell differentiation during ocular lens development. The vertebrate lens initiates from a placodal thickening of the surface ectoderm, adjacent to the optic vesicle. This lens placode invaginates into a pit, and then vesicle, within the space vacated by the forming optic cup (reviewed in Lang, 2004; McAvoy et al., 1999). As the lens vesicle forms, it separates from the surface ectoderm, which later gives rise to the cornea. Within the hollow lens vesicle, anterior–posterior compartmentalization occurs as posterior progenitor cells, closest to the forming retina, exit the cell cycle and differentiate as primary lens fibers. Thus, proliferative lens progenitor cells are progressively sequestered in the anterior vesicle, where they coalesce into an epithelial growth zone that persists into adulthood. Once formed, the anterior epithelial layer (AEL) produces secondary lens fibers by peripheral cell movement, through a germative zone, and into the transition zone at the lens equator. Cells in the transition zone become postmitotic, differentiate, move centrally and elongate as mature fiber cells.

The transcription factors *Pax6*, *Sox1*, *Sox2*, *Prox1*, *Foxe3*, *Pitx3*, *AP2α*, and *Maf* are critically required for lens formation, and constitute a partial lens regulatory gene network (reviewed in Cvekl and Duncan, 2007; Graw, 2003; Lang, 2004). Importantly, mutations in several of these human genes cause anophthalmia, microphthalmia, Peter's Anomaly and/or aphakia, wherein the lens is absent or defective by birth (Ashery-Padan et al., 2000; Blixt et al., 2000; Glaser et al., 1994; Grimm et al., 1998; Kim et al., 1999; Medina-Martinez et al., 2005; Rieger et al., 2001; Semina et al., 1998; Semina et al., 1997). But, signal transduction pathways are equally important during lens development. For example *FGF*, *BMP*, *Wnt* and *Notch* signaling regulate key aspects of lens formation (Beebe et al., 2004; Cain et al., 2008; Faber et al., 2002; Jia et al., 2007; Lovicu and McAvoy, 2001; Ogino et al., 2008; Robinson, 2006; Rowan et al., 2008; Song et al., 2007; Zhao et al., 2008).

The *Notch* pathway functions in the lens, as it does throughout the body, to transduce cell contact-mediated communication. The ligands are of two types: *Delta*/*Deltalike* (DLL) or *Jagged*/*Serrate*/*Lag2*, which bind a *Notch* receptor via their extracellular DSL domain. There are four *Notch* receptor genes in mammals (reviewed in Gridley, 2003; Louvi and Artavanis-Tsakonas, 2006), and whether one or multiple *Notch* genes act during lens formation remains unclear. Nevertheless, upon ligand–receptor binding a series of proteolytic cleavages are triggered that release the intracellular domain ($Notch^{IC}$), allowing it to form a nuclear complex with the Su(H)/Rbpj and mastermind/MAML transcription factors. This complex then activates the transcription of downstream effector genes (reviewed in Ilagan and Kopan, 2007; Kopan, 2002; Louvi and Artavanis-Tsakonas, 2006). Major targets of *Notch* signaling are the *hairy-E(spl)/Hes* transcriptional repressors (reviewed in Davis and Turner, 2001; Kageyama et al., 2007). While invertebrates (e.g. *Drosophila*), encode essentially one gene for each part of the *Notch* pathway, vertebrates have multiple paralogues for nearly every component. This allows additional levels of regulatory

* Corresponding author. Fax: +1 513 636 4317.
E-mail address: nadean.brown@cchmc.org (N.L. Brown).

0012-1606/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2009.01.015

complexity during vertebrate development, such that different ligands may transduce information about distinct cellular processes to the same receptor, or one ligand may send multiple signals by binding and activating more than one receptor. Multiple ligands, receptors and downstream effectors simultaneously acting within a single cell or cell type can impose additional layers of signal information. Finally, the *Notch* pathway transduces two main types of signals, a classical lateral inhibition signal (Cabrera, 1990; Simpson, 1990), wherein adjacent cells compete for ligand expression, or a lateral induction signal (Eddison et al., 2000; Lewis, 1998), in which a group of cells exhibit cooperative ligand expression.

In the lens, *Notch* signaling has multiple roles. In the frog optic vesicle, Delta1 activates Notch in the lens placode, thereby triggering activation of the *Foxe3* lens enhancer through binding of a Notch^{IC}–Rbpj–MAML complex to DNA, adjacent to a site of Otx2 protein binding (Ogino et al., 2008). This synergistic activation of *Foxe3* is crucial for lens vesicle formation and growth (Blixt et al., 2000). Meanwhile in the mouse, lens-specific deletion of *Rbpj*, or misexpression of the *Notch1^{IC}* demonstrate that there are other, late *Rbpj*-dependent functions for *Notch* signaling, during primary fiber cell genesis, lens progenitor cell growth in the AEL and secondary fiber cell differentiation (Jia et al., 2007; Rowan et al., 2008). As primary fiber cells differentiate, one *Notch* ligand, *Jagged 1* (*Jag1*), becomes localized posteriorly. Then, during secondary fiber formation *Jag1* expression is confined to transition zone cells, and the anterior side of extended fiber cells, at the border with the AEL (Rowan et al., 2008). These data suggest *Jag1* may act both in transition cells to regulate secondary fiber cell formation and in fiber cells to signal *Notch–Rbpj–Hes1*+ progenitor cells in the AEL (Rowan et al., 2008).

To investigate these ideas further, we directly tested the requirements for *Jag1* during mammalian lens formation. Germline deleted *Jag1* mutants die before lens formation is underway, but hemizygous mice have uncharacterized eye defects (Xue et al., 1999). Therefore, to determine when and how this ligand acts during lens development, we used a Cre–Lox strategy to selectively remove *Jag1*. We observed that although *Jag1* is necessary for aspects of primary fiber cell formation, *Jag1* plays a major role in the AEL and transition zone formation, since both compartments fail to become established in lens-specific deletion mutants. Without proper tissue compartmentalization, the lens is microphakic by birth, and contains only a handful of β-Crystallin+ cells. *Jag1* conditionally mutant adult eyes lacked lenses, anterior chambers and pupillary openings. These data support a model that *Jag1–Notch* signaling is essential for lens growth and fiber cell differentiation. Furthermore, during lens development *Jag1* appears to transduce multiple signals that are spatially and temporally restricted, and mediates distinct developmental events.

Materials and methods

Animals

Jagged 1^{tm1JLew} mice (*Jag1*^{CKO}) were generated by Brooker et al., maintained on a C57BL/6 background and genotyped as described (Brooker et al., 2006). *Rbpj*^{tm1Hon} mice (*Rbpj*^{CKO}) were generated by Han et al., maintained on a 129/SvJ background and genotyped as described (Han et al., 2002). The abbreviation CKO indicates a "conditional knock-out" allele. *Le-Cre* mice were generated by Ashery-Padan et al., maintained on an FVB/N background and PCR genotyped as described (Ashery-Padan et al., 2000). Images of adult heads were captured with a Leica dissecting microscope and Optronics digital camera.

Tissue analyses

Embryonic and postnatal tissues were fixed in 4% paraformaldehyde/PBS for 1 h at 4 °C, processed through a sucrose/PBS series, cryoembedded and sectioned. Primary antibodies used were anti-BrdU (Serotec clone BU1/75 1:500), anti-cleaved PARP (Cell Signaling 1:500), anti-Cyclin D1 (Neomarkers SP4 1:100; or Santa Cruz 72-13G 1:500), anti-Cyclin D2 (Santa Cruz 34B1-3 1:200), anti-E cadherin (Zymed ECCD-2 1:500), anti-Foxe3 (gift from Peter Carlsson 1:1000), anti-β-Crystallin (gift from Richard Lang 1:8000), anti-γ-Crystallin (Santa Cruz 1:1000), anti-GFP (Molecular Probes 1:1000), anti-Hes1 (1:1000), anti-Jag1 (Santa Cruz 1:1000), anti-p27^{Kip1}(BD Laboratories Clone 57 1:100), anti-p57^{Kip2} (Abcam 1:200), anti-Pax6 (gift from Grant Mastick 1:1000), anti-Prox1 (Covance 1:1000), anti-Pitx3 (gift from Marten Smidt 1:1000), anti-Six3 (gift from Guillermo Oliver 1:1000), anti-Sox1 (Affinity BioReagents 1:500), and anti-Sox2 (Chemicon 1:500), following (Lee et al., 2005). Secondary antibodies were directly conjugated to Alexa Fluor 488, Alexa Fluor 594 (Molecular Probes) or biotinylated (Jackson Immunologicals) and sequentially labeled with streptavidin Alexa 488 or 594 (Molecular Probes). Microscopic imaging was performed on a Zeiss fluorescent microscope with a Zeiss camera and Apotome deconvolution device. Whole-mount or cryosection in situ hybridization was performed as described (Brown et al., 1998) using a *Jag1* digoxygenin-labeled

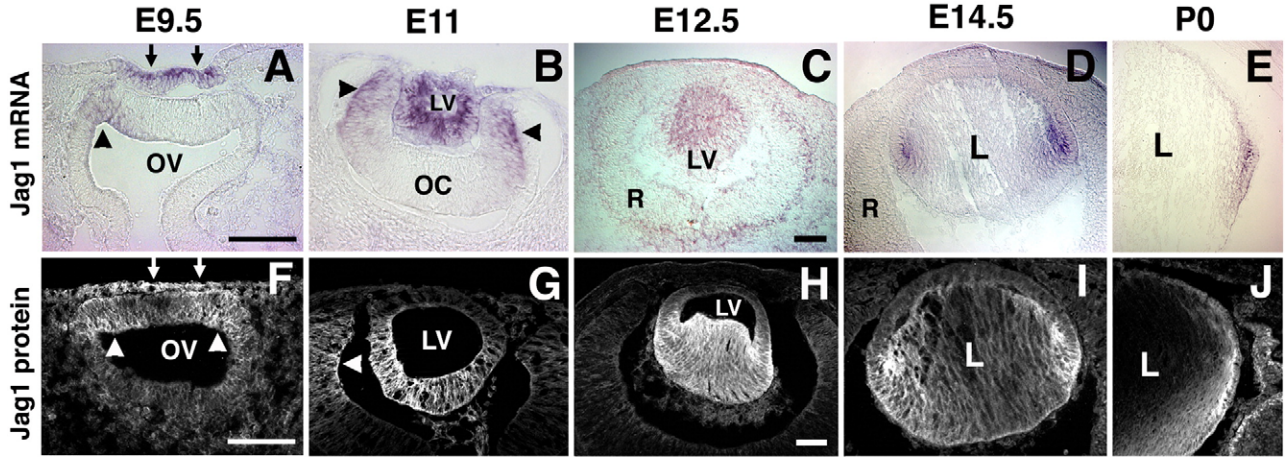

Fig. 1. Spatiotemporal expression of *Jag1* mRNA and protein during mouse lens formation. In situ hybridization to detect *Jag1* mRNA (A–E) and antibody labeling to visualize *Jag1* protein (F–J) from E9.5 to birth. *Jag1* is expressed in the lens placode beginning around E9.5 (arrows in A, F) and throughout the E10.5–11.5 lens vesicle (B, G). At these early ages *Jag1* is also present in the distal optic vesicle and cup (arrowheads in A, B, F, G). Between E11.5 and E12.5, *Jag1* expression becomes localized to the posterior lens vesicle, where primary fiber cells differentiate (G, H). From E14.5 to at least P3, *Jag1* mRNA and protein are predominantly expressed at the equatorial transition zone (D–E, I–J and data not shown). At these older ages, protein expression is broader than that of mRNA (compare E and J). Anterior is up in all panels; bar in A, C, F, H = 20 μm. OV = optic vesicle, OC = optic cup, LV = lens vesicle, L = lens, and R = retina.

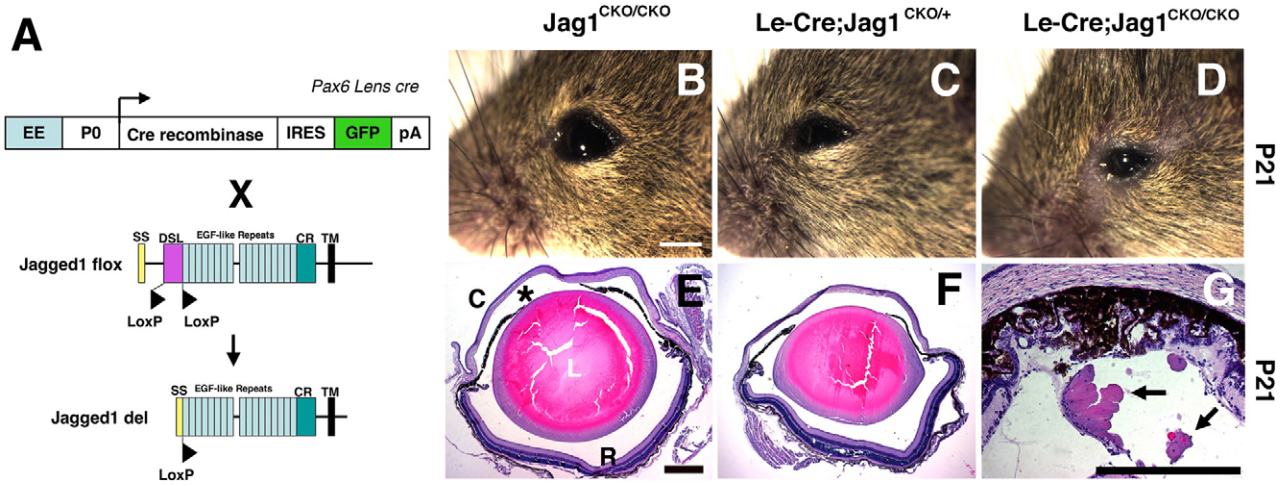

Fig. 2. Adult phenotypes of Le-Cre;*Jag1*$^{CKO/CKO}$ eyes. (A) Conditional deletion strategy to create *Le-Cre; Jag1*$^{CKO/CKO}$ lens mutants. (B–D) *Le-Cre; Jag1*$^{CKO/+}$ adult eyes are grossly indistinguishable from *Jag1*$^{CKO/CKO}$ littermate controls ($n \geq 3$ per genotype). However, *Le-Cre; Jag1*$^{CKO/CKO}$ mutants have bilateral microphthalmia with missing fur and whiskers around the eye and snout ($n > 3$). Mutant eyes lack pupillary openings (not shown). (E–G) Histologic sections of these P21 eyes show slight reduction in the size of *Le-Cre; Jag1*$^{CKO/+}$ lenses. *Le-Cre; Jag1*$^{CKO/CKO}$ eyes have essentially no lenses, although cellular debris resembling lens fiber cells could be found in some sections (arrows in G). Note complete absence of the anterior chamber and abnormally folded ciliary body/iris tissue that contains black pigment granules. Rostral is left in B–D; anterior up in E–G; bar = 500 μm in B; 5 μm in E,G. EE = ectoderm enhancer, P0 = Pax6 promoter, pA = poly A sequence, SS = signal sequence, DSL = Delta/Serrate/Lag domain, CR = cysteine-rich domain, TM = transmembrane domain, L = lens, R = retina, C = cornea, and * = anterior chamber.

antisense riboprobe. For S-phase analyses, BrdU (Sigma) was injected intraperitoneally as described in Mastick and Andrews (2001) and animals sacrificed 1.5 h later for tissue processing that included 2 N hydrochloric acid treatment of sections prior to antibody staining. Standard histology on paraffin embedded sections was also performed. Images were processed using Axiovision (v5.0) and Adobe Photoshop software (v7.0) and electronically adjusted for brightness, contrast and pseudocoloring.

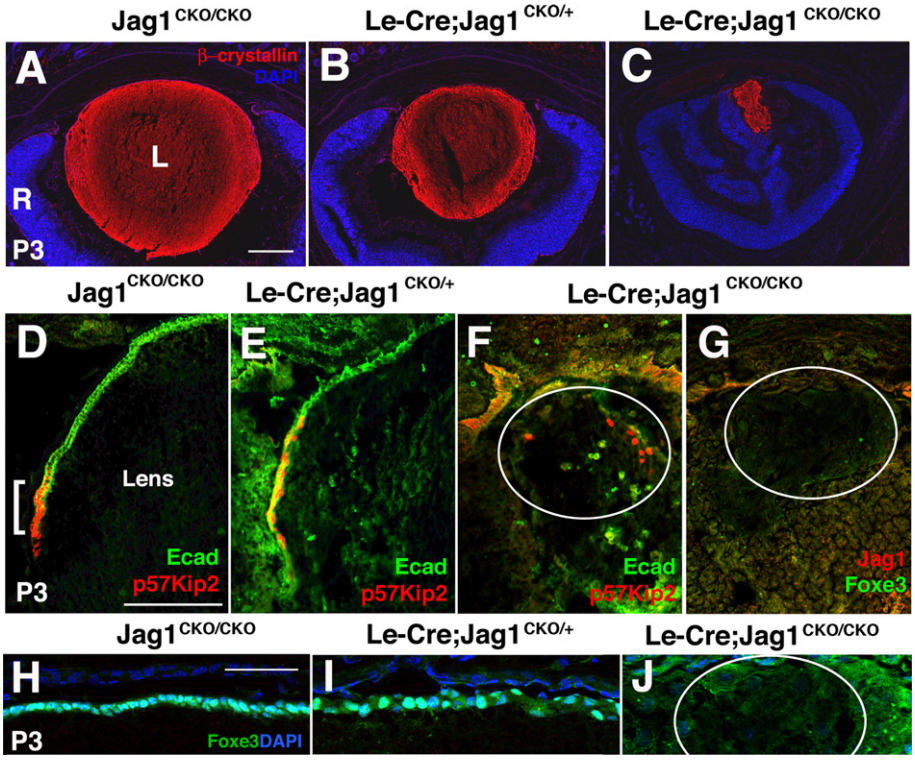

Fig. 3. Postnatal *Jag1* lens mutants have a profound loss of lens fibers and no AEL or transition zone. All panels contain sections of postnatal (P3) eyes. (A–C) β-Crystallin expression in lens fiber cells (red) highlights the smaller size of *Le-Cre; Jag1*$^{CKO/+}$ lenses (B) and profound loss of this tissue in *Le-Cre; Jag1*$^{CKO/CKO}$ eyes (C). In the near absence of a lens, the retina (R, in blue) is abnormally folded. (D–F) p57^{Kip2}-E-cadherin (Ecad) double antibody labeling delineates the equatorial transition zone, where anterior progenitor cells (in green) exit mitosis (in red) to differentiate as secondary fiber cells. Normally there is minimal overlap of these two markers at the equator (bracket in D). In *Le-Cre; Jag1*$^{CKO/+}$ eyes, the p57^{Kip2}+ domain is unusually elongated around the periphery, with inappropriate expression of both markers (E). However, in *Le-Cre; Jag1*$^{CKO/CKO}$ mutants, only sparse, randomly positioned, p57+ or Ecad+ cells were identifiable (white circle marks edges of lens tissue) (F). The p57^{Kip2}+ Ecad+ cells above the lens are in the ciliary body or cornea. (G) Foxe3–Jag1 double antibody labeling confirms the loss of AEL and transition zone compartments, and removal of Jag1 protein from the lens in conditional mutants. F and G are sections nearby to that in C, note differences in magnification. (H–J) AEL lens progenitor cell nuclei double labeled with anti-Foxe3 and DAPI. Normally these cells are tightly organized into a monolayer epithelium (H). But, *Le-Cre; Jag1*$^{CKO/+}$ lenses the arrangement of Foxe3+ progenitor cell nuclei is abnormal. These cells are completely missing from the postnatal *Jag1* mutant lens (circle in J). $n = 3$ animals per genotype and marker. Anterior is up in all panels; bar in A–C = 5 μm, D–G = 50 μm, and H–J = 100 μm. L = lens, R = retina.

Cell counting

Tissue sections, separated by at least 60 μm, were antibody-stained and counted using Axiovision software. Three or more animals were analyzed per genotype and age and at least two independent sections through the center of the lens per animal quantified. Labeling indices were generated by dividing the number of antibody-positive cells by total DAPI-labeled nuclei, and Instat (v3.0) software used to perform ANOVA plus a Bonferroni posthoc test to determine *p* values.

Results

Jagged 1 mRNA and protein expression during prenatal lens development

Although basic expression of *Jag1* in the developing vertebrate eye is known (Bao and Cepko, 1997; Jia et al., 2007), its expression pattern across the key stages of lens development is uncharacterized. Therefore, we examined *Jag1* mRNA and protein expression from embryonic day 9.5 (E9.5) to postnatal day 3 (P3), by both in situ hybridization and anti-Jag1 staining (Fig. 1). From E9.5–10.5, *Jag1* mRNA and protein are specifically expressed in lens placode (arrows in Figs. 1A, F), lens pit (not shown), and distal optic vesicle cells (arrowheads in Figs. 1A, F). When the lens vesicle pinches off from the surface ectoderm around E11, *Jag1* mRNA and protein are abundant throughout vesicle cells (Figs. 1B, G). But once primary fiber differentiation commences between E11.5 and E12.5, *Jag1* mRNA and protein become sequestered in posterior lens vesicle cells (Figs. 1B, C, G, H). Interestingly after E12.5, *Jag1* mRNA and protein were no longer detectable in the peripheral optic cup.

In the E14.5 lens, *Jag1* mRNA is abundant in equatorial cells (Fig. 1D) once the AEL, transition zone, and fiber cell compartments are fully established. Likewise, Jag1 protein is observed in transition zone cell membranes, and along the anterior side of fiber cells, where they are in close contact with AEL progenitor cells (Fig. 1I). These expression patterns for *Jag1* mRNA and protein persist beyond birth (Figs. 1E, J) to at least P3 (not shown). One consistent difference between *Jag1* mRNA and protein patterns, is a broader protein domain that includes newly born secondary fiber cells (compare Figs. 1I, J to D, E), suggesting that either *Jag1* mRNA is very tightly regulated, or Jag1 protein persists longer than the mRNA.

Conditional deletion of Jag1 during lens development causes aphakia

Previously, we used the Le-Cre driver (Ashery-Padan et al., 2000) to delete the nuclear *Notch* effector *Rbpj* during mouse lens development (Rowan et al., 2008). Loss of *Rbpj* causes adult eyes to be

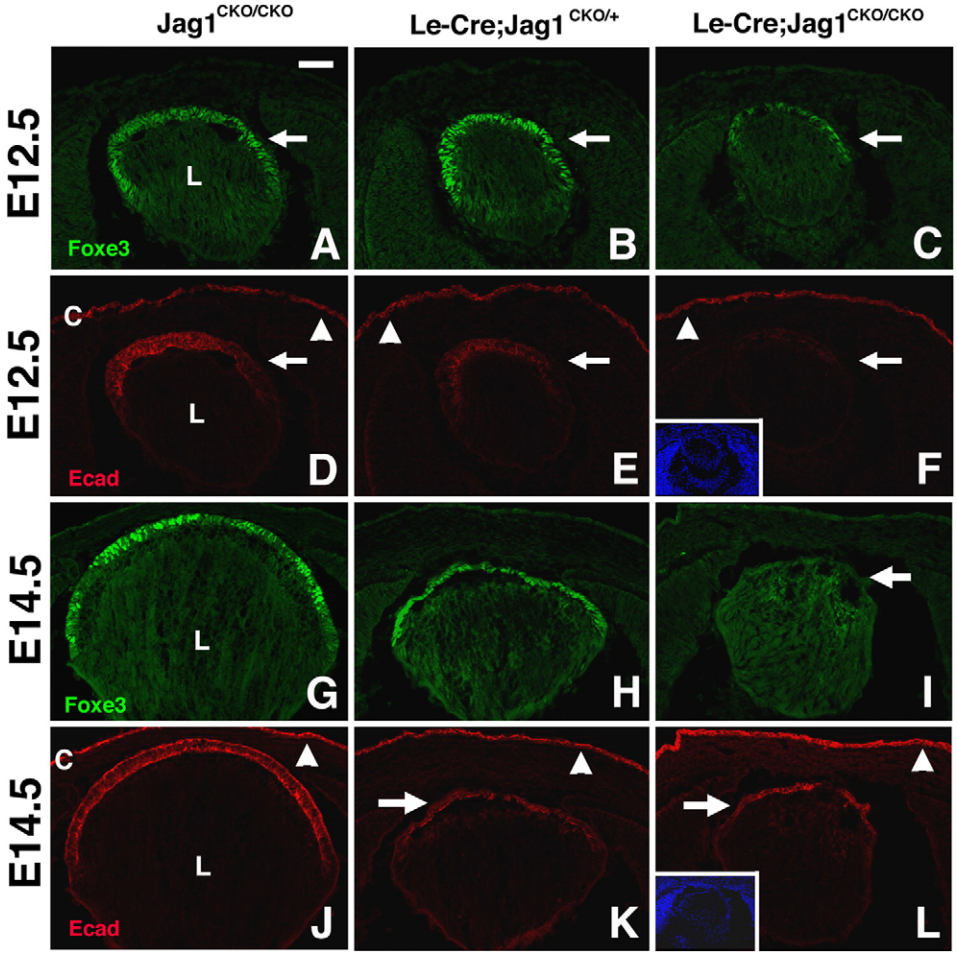

Fig. 4. Anterior epithelial lens cells critically require *Jag1*. (A–F) E12.5 lens sections double labeled with Foxe3 (green) and Ecad (red). The expression of both markers is restricted to lens progenitor cells in the anterior epithelial layer (AEL). In *Le-Cre;Jag1*$^{CKO/+}$ and *Le-Cre;Jag1*$^{CKO/CKO}$ eyes, there is a progressive loss of both Foxe3 and Ecad in the anterior lens (arrows in all panels, $n=4$ embryos/genotype). Note Ecad expression in the forming cornea appears unaffected (arrowheads in D–F, J–L). (G–L) At E14.5, the smaller size of *Le-Cre;Jag1*$^{CKO/+}$ and *Le-Cre;Jag1*$^{CKO/CKO}$ lenses is apparent, along with dramatic reduction of Foxe3 and Ecad expression. For each marker, imaging exposure time was held constant among genotypes at each age. Among four E14.5 *Jag1* lens mutant embryos, half had no anterior Foxe3+ cells (arrow in I), and the rest a handful of Foxe3+ AEL cells (not shown). Insets in panels F and L show that DAPI-labeled fiber cell nuclei are appropriately positioned in mutant lenses. Low level, patchy fluorescence in the fiber cell compartment sometimes occurred (H, I), but was not meaningful since control sections lacking primary antibody exhibit the same pattern. Ecad expression in the AEL (arrows) is abnormal in both K and L. Anterior is up in all panels; bar=20 μm. L = lens, C = cornea.

microphthalmic, with total loss of the pupillary opening and the anterior chamber, and dramatic reduction of lens tissue (Rowan et al., 2008). Using the same cre–lox deletion strategy (Fig. 2A), Le-Cre; *Jag1*$^{CKO/CKO}$ and Le-Cre; *Jag1*$^{CKO/+}$ P21 mice were generated in expected Medelian ratios. Le-Cre;*Jag1*$^{CKO/CKO}$ mutants are devoid of fur around severely microphthalmic eyes that lack pupillary openings (Fig. 2D and data not shown). Heterozygotes have less severely reduced eyes (Fig. 2C). Histologic cross sections show that progressive loss of the lens correlates with reduction of *Jag1* gene dosage (compare Figs. 2E–G; $n=3$/genotype). Adult Le-Cre;*Jag1*$^{CKO/CKO}$ eyes are missing lenses and anterior chambers, accompanied by expansion of iris and/or ciliary body tissue (Fig. 2G). Upon close examination, small clumps of lens fiber-like cells are identifiable in some sections of *Jag1* conditionally mutant eyes (arrows in Fig. 2G).

The adult phenotypes of Le-Cre;*Jag1*$^{CKO/CKO}$ and Le-Cre;*Rbpj*$^{CKO/CKO}$ eyes are strikingly similar, but *Jag1* conditional mutants are more severe (compare Fig. 2G to Fig. 1E of Rowan et al., 2008). To understand when lens formation goes awry in Le-Cre;*Jag1*$^{CKO/CKO}$ eyes and whether developmental defects are the same or different from those in *Rbpj* mutant lenses, we next analyzed P3 *Jag1* conditional mutants. At this age, lens tissue could be discerned in sections through Le-Cre;*Jag1*$^{CKO/CKO}$eyes, with a small number of β-Crystallin+ lens fibers (Figs. 3A–C). We also observed that Le-Cre; *Jag1*$^{CKO/+}$ littermates have smaller sized lenses than controls. This loss of Crystallin+ fibers was also obvious at E18.5 (Supp Figs. 1A–C). To determine if the remnant lenses in *Jag1* conditional mutants contain distinct AEL, transition zone and fiber cell regions, adjacent sections were colabeled with anti-Ecadherin (Ecad) and anti-p57/Kip2, to mark the AEL and transition zone cells, respectively (Fig. 3D). At the boundary of these compartments, a small number of progenitor cells normally coexpress both markers (shown in yellow in the bracketed region in Fig. 3D). By contrast, Le-Cre;*Jag1*$^{CKO/+}$ eyes have more peripheral cells coexpressing Ecad and p57/Kip2 (Fig. 3E). Strikingly, Le-Cre;*Jag1*$^{CKO/CKO}$ eyes lack both an AEL and transition zone, with only a few random cells expressing either marker (circled area of Fig. 3F). The breakdown of these peripheral compartments was also observed at E18.5 (Supp Figs. 2D–F). To confirm the loss of the AEL, we analyzed Foxe3 expression, which is expressed by lens progenitor cells from E9.5 to adulthood, where it regulates proliferation (Blixt et al., 2007; Blixt et al., 2000). We found E18.5-P3 Le-Cre;*Jag1*$^{CKO/CKO}$ eyes to be essentially devoid of Foxe3 expression (Figs. 3G, J, Supp Fig 1I, $n=3$ animals). At E18.5, only small patches of nonnuclear expression were observable in the absence of *Jag1* (Supp Fig 1I). At high magnification of P3 lenses, the normal, orderly arrangement of AEL cells within a monolayer is apparent (Fig. 3H). But, in Le-Cre; *Jag1*$^{CKO/+}$ lenses, Foxe3+ cells were irregularly arranged (Fig 3I). Colabeling with anti-Jag1 confirmed the loss of protein expression in P3 *Jag1* lens mutants (compare Fig. 3G to Fig. 1J). We conclude that the AEL and transition zone compartments have broken down by birth in Le-Cre;*Jag1*$^{CKO/CKO}$ eyes, a more severe phenotype than that of Le-Cre;*Rbpj*$^{CKO/CKO}$ lenses (compare Fig. 3 to Fig. 8 in Rowan et al., 2008).

Jag1 function is critical for establishment and maintenance of the anterior epithelial layer (AEL)

The small number of β-Crystallin+ fibers and loss of the AEL and transition zone at birth suggests that without *Jag1* function, lens development breaks down soon after primary fiber cells form. Therefore we assayed the expression of two lens progenitor cell markers: Foxe3 (green) and E-cadherin (Ecad, red) from E12.5 to E14.5, when the AEL compartment becomes established (Blixt et al., 2000; Medina-Martinez et al., 2005; Xu et al., 2002). Foxe3 specifically marks lens progenitor cells, while Ecad is expressed by both lens progenitor and corneal epithelial cells (arrowheads in Figs. 4D–F, J–L). Interestingly, both proteins become inappropriately downregulated in the AEL of Le-Cre;*Jag1*$^{CKO/+}$ and Le-Cre;*Jag1*$^{CKO/CKO}$ embryos ($n\geq 3$/genotype). Already at E12.5 a significant loss of Foxe3 expression is apparent in Le-Cre;*Jag1*$^{CKO/CKO}$ lenses (Figs. 4C, and data not shown). Simultaneously, the Ecad domain is reduced in E12.5 Le-Cre;*Jag1*$^{CKO/+}$, or completely missing from 50% of Le-Cre;*Jag1*$^{CKO/CKO}$ lenses (Figs 4E, F; $n=4$ embryos per genotype). Although the Le-Cre driver removes *Jag1* in both developing lens and corneal epithelium (Ashery-Padan et al., 2000), we find Ecad expression is only downregulated in the lens.

Loss of Foxe3 and Ecad expression persists at E14.5 (Figs. 4G–L), where 50% of Le-Cre;*Jag1*$^{CKO/CKO}$ eyes examined have no Foxe3 expression (Fig. 4I), and the rest have sporadic, rare Foxe3+ nuclei ($n=4$ embryos per genotype). Similarly, the Ecad lens domain is abnormal in both E14.5 *Jag1* heterozygous and homozygous mutants (Figs. 4K, L), with Ecad expression in the cornea unaffected (arrowheads). However, not all AEL characteristics are lost, since we observed Pax6 and Sox2 expression at E12–E14.5 are unaffected ($n=3$/age and genotype; data not shown). We conclude that in the absence of *Jag1*, embryonic lens progenitor cells accumulate in the AEL, but are unable to maintain all of their epithelial features.

Jag1 function is primarily required during secondary lens fiber cell differentiation

Dramatic reduction in Foxe3 and Ecad expression may reflect a loss of lens progenitor cells by either apoptotic cell death or premature differentiation. As is the case for Le-Cre;*Rbpj*$^{CKO/CKO}$ mutants (Jia et al., 2007; Rowan et al., 2008), we assayed cleaved PARP expression from E10.5 to P3, and found no increase in apoptosis (not shown). *Foxe3* is exclusively found in lens progenitor cells and when mutated, causes premature fiber cell differentiation (Blixt et al., 2000; Medina-Martinez et al., 2005; Valleix et al., 2006). Therefore, we determined the percentage of Foxe3-negative cells at E11, E12.5 and E14.5 (Figs. 5A, G–I), as a substitute for quantifying lens fibers directly. Interestingly, at E11 and E14.5, fiber cells are significantly increased. Because removal of *Rbpj* during this period of lens development produced the same outcome (Jia et al., 2007; Rowan et al., 2008), we conclude that *Jag1–Notch* signaling regulates aspects of primary and secondary fiber cell differentiation. However, neither β- or γ-Crystallin are expressed precociously or inappropriately in the AEL of E9.5 to E14.5 *Jag1* mutant lenses (Suppl. Fig. 2 and data not shown).

Jag1–Notch signaling regulates lens progenitor cell cycle progression

Since diminished Foxe3 and Ecad AEL expression is followed by loss of this lens compartment, we wished to understand better when and to what extent cell proliferation is affected. First, we quantified the percentage of S-phase progenitor cells in BrdU pulsed-labeled embryos and found significant loss of BrdU+ cells during primary and early secondary fiber cell genesis (Figs. 5B, J–L). This could be generally correlated with significant reduction in the percentage of CyclinD1+ cells in E10.5 and E14.5 Le-Cre;*Jag1*$^{CKO/CKO}$ eyes (Figs. 5C, M–O). But, Cyclin D1-expressing cells rebounded beyond wild type numbers only at E12.5 (Fig. 5C). Interestingly, in Le-Cre;*Rbpj*$^{CKO/CKO}$ lenses, a similar but less robust phenomenon was observed for Cyclin D2 expression (Fig. 6G of Rowan et al., 2008). Paradoxically, in Jag1 conditional lens mutants, the number and pattern of Cyclin D2+ cells was unaffected (Fig. 5D), suggesting that *Jag1–Notch* signaling specifically regulates an aspect of Cyclin D1 expression, while Cyclin D2 is regulated by the *Notch* pathway independent of *Jag1*. Interestingly, the expression of the CKI p27^{Kip1} was profoundly reduced at E12.5 in Le-Cre;*Jag1*$^{CKO/CKO}$ lenses (Fig. 5E). This is similar to the reduced numbers of these cells in Le-Cre;*Rbpj*$^{CKO/CKO}$ eyes (Fig. 6K of Rowan et al., 2008), but the temporal kinetics differ between the two lens mutants. Finally, p57^{Kip2} expressing cells were quantified, and found to be significantly increased only at E14.5 (Fig. 5F). In E14.5 Le-Cre;*Jag1*$^{CKO/CKO}$ eyes,

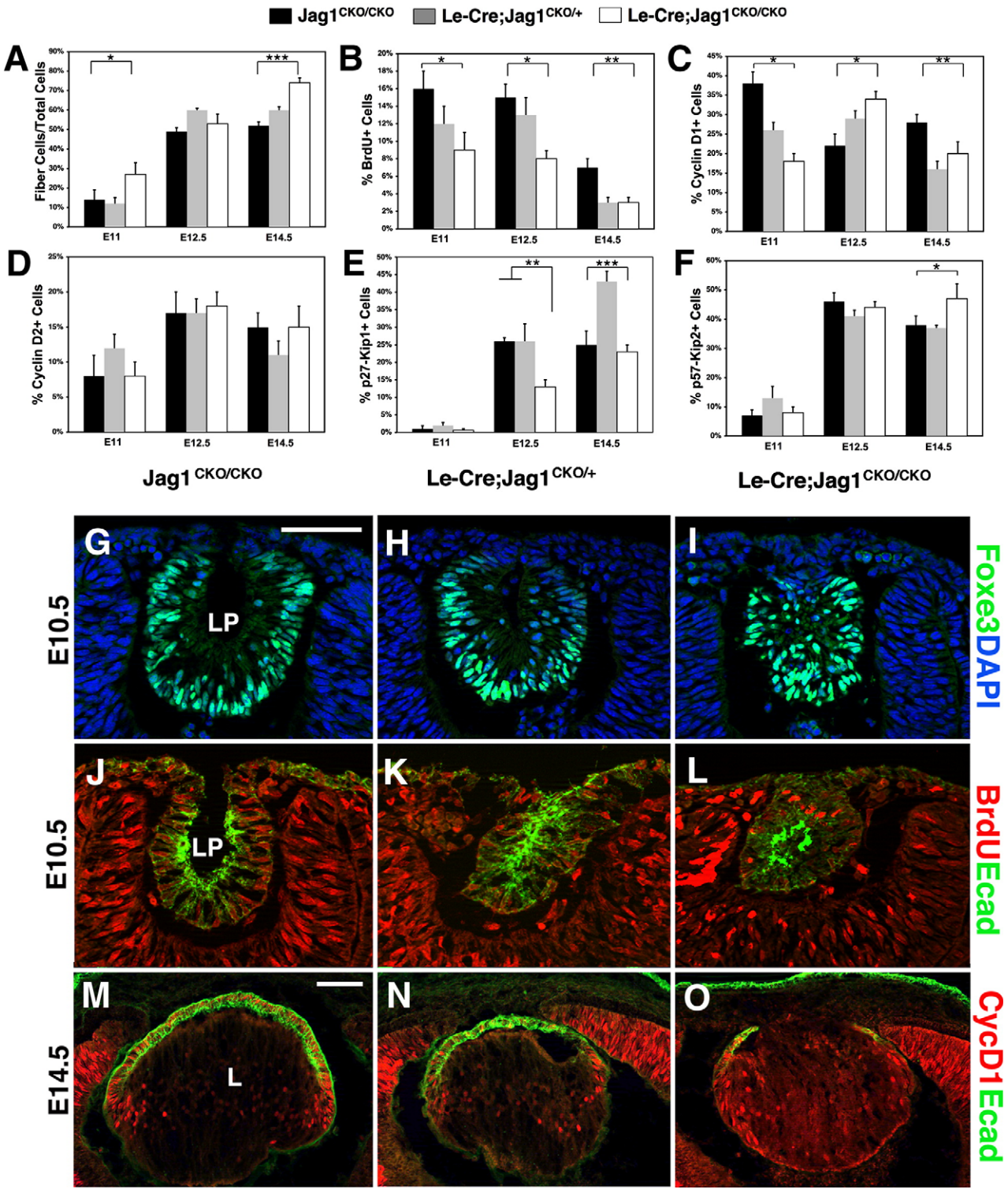

Fig. 5. Loss of *Jag1* affects both lens progenitor cell proliferation and differentiation. (A) The percentage of fiber cells (Foxe3-negative/total cells) is increased at E10.5 and E14.5 *Le-Cre; Jag1*$^{CKO/CKO}$ lenses. (B–L) Progenitor cell cycle markers in *Jag1* lens mutants. (B) BrdU+ cells are significantly decreased at all ages analyzed. (C) Cyclin D1+ cells are lost in E10.5 and E14.5 *Le-Cre;Jag1*$^{CKO/CKO}$ lenses, but rebound above normal levels at E12.5. (D) By contrast, Cyclin D2+ cells are not significantly unaffected. (E) There are significantly fewer p27^{Kip1}+ cells during secondary fiber cell genesis in E12.5–E14.5 *Le-Cre;Jag1*$^{CKO/CKO}$ eyes. (F) The percentage of p57^{Kip2}+ cells correlates with increased fiber cells only at E14.5. G–L) Example Foxe3/DAPI (G–I) BrdU/Ecad (J–L) and CyclinD1/Ecad (M–O) labeled lenses used for quantification in A–C (DAPI channel omitted in J–O) showing obvious reduction of BrdU+ and Cyclin D1+ nuclei in I and L respectively. Note the split Ecad expression domain in L, with both CyclinD1 and Ecad expression missing from the central AEL. Bar graphs show mean±s.e.m., *$p<0.05$, **$p<0.01$, and ***$p<0.001$. LP = lens pit; L = lens; bar in panels G, M=20 μm.

Cyclin D2 and p57^{Kip2} expression were properly localized to the lens equator (not shown), suggesting that in the absence of *Jag1*, the transition zone, like the AEL, initiates formation but cannot be sustained.

Jag1–Notch signaling does not feedback on Jag1 expression

Although the mutant phenotypes of *Jag1* and *Rbpj* conditional lens mutants are grossly identical, the loss of *Jag1* is more severe and

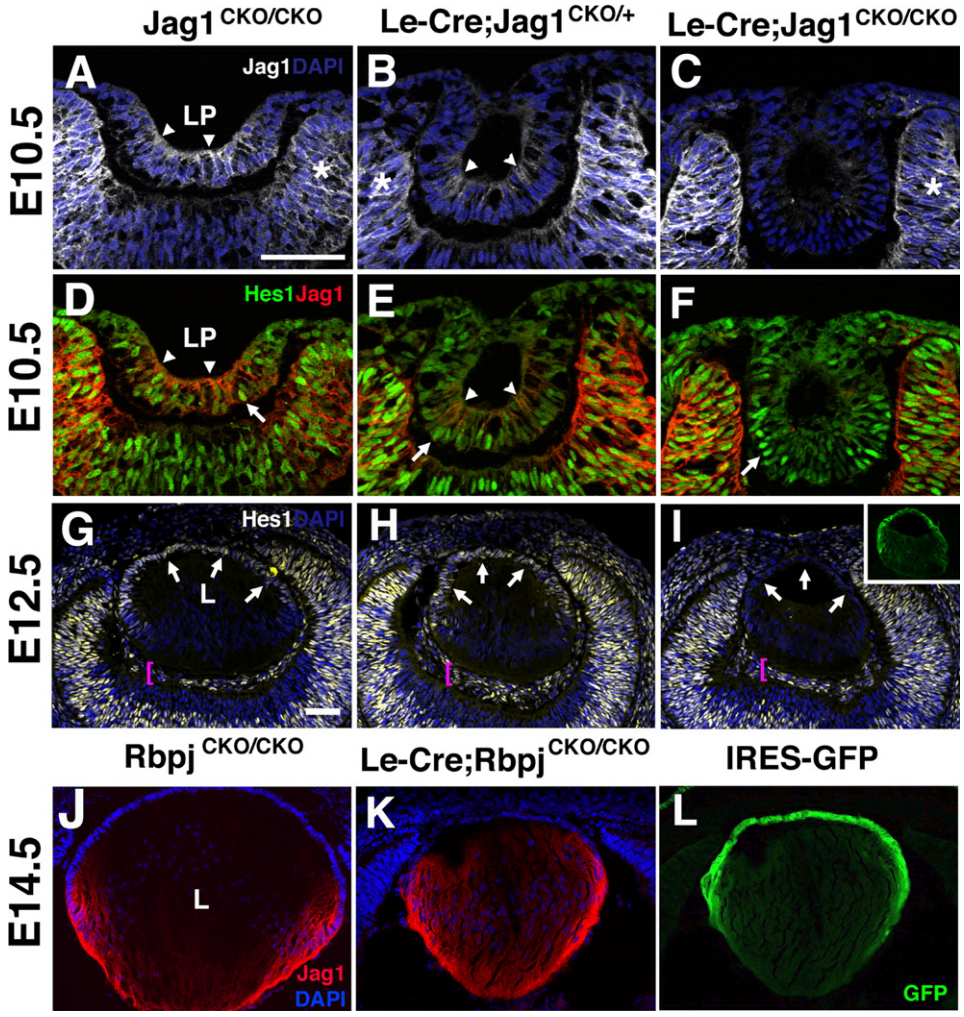

Fig. 6. *Jag1–Notch* signaling in the lens is not bidirectional. (A–C) As early as E10.5, Jag1 protein is reduced in *Le-Cre;Jag1*$^{CKO/+}$ and total loss in *Le-Cre;Jag1*$^{CKO/CKO}$ lens pits (arrowheads point to Jag1+ cell membranes in white; $n=3$ embryos/genotype). However, Jag1 in the distal optic cup (denoted by asterisks) is unaffected. (D–E) The Notch pathway effector Hes1 (green) is not obviously affected in E10.5 *Le-Cre;Jag1*$^{CKO/+}$ or *Le-Cre;Jag1*$^{CKO/CKO}$ mutants (arrows point to Hes1+ nuclei, arrowheads point to Jag1 in cell membranes). (G–I) By E12.5, Hes1 is totally missing from *Jag1* mutant lenses (arrows in G–I, $n=3$ embryos/genotype), two days earlier than in *Le-Cre;Rbpj*$^{CKO/CKO}$ (Fig. 1G of Rowan et al., 2008). Fuchsia brackets denote unaffected Hes1 expression in the hyaloid vasculature immediately posterior to the lens. J–L) Importantly, Jag1 expression appears normal in *Le-Cre;Rbpj*$^{CKO/CKO}$ lenses (compare K to J; $n=3$ embryos/genotype). GFP expression in the inset in I and in panel L indicates cells with Cre activity. LP = lens pit; L = lens; bar in A, G = 20 μm.

onsets at an earlier stage of lens development. For example, *Rbpj* conditional lens mutants do not exhibit the same dramatic changes in Foxe3 or Ecad expression at E12.5–E14.5 (Fig. 6 of Rowan et al., 2008). Moreover, different shifts in markers of cell proliferation versus cell cycle exit were found between these two mutants. Thus, we wished to explore the molecular epistatic relationships among several *Notch* pathway components to determine if there is feedback upon *Jag1* expression and how early Jag1 is deleted during lens development. For the latter question, anti-Jag1 immunolabeling demonstrated efficient elimination of the targeted protein in the mouse inner ear (Brooker et al., 2006), so we also used this approach here. In E10.5 sections of embryonic eyes from Le-Cre;*Jag1*$^{CKO/+}$ intercrosses double labeled with anti-Jag1 and anti-Hes1, Jag1 protein was completely gone from the lens pit of Le-Cre;*Jag1*$^{CKO/CKO}$ embryos (Fig. 6C), and reduced in those of Le-Cre;*Jag1*$^{CKO/+}$ embryos (Fig. 6B; $n=3$ embryos per genotype). The adjacent optic cup domain was unaffected (asterisks in Figs. 6A–C). Although Hes1 expression appears normal in E10.5 *Jag1* lens mutants (arrow in Fig. 6F), by E12.5, it was completely missing from Le-Cre;*Jag1*$^{CKO/CKO}$ lens vesicles. Thus, the loss of Hes1 protein in *Jag1* lens mutants occurs two days earlier than in *Rbpj* lens mutants.

Next, we reciprocally examined *Jag1* expression in Le-Cre; *Rbpj*$^{CKO/+}$ and Le-Cre;*Rbpj*$^{CKO/CKO}$ mutants. From E10.5 to E15.5 no obvious change in Jag1 expression was found (Figs 6J, K and data not shown; $n=3/3$ mutants). To independently confirm this outcome, we also scrutinized Jag1 expression in E10.5 or E13.5 *Hes1* germline mutants, but could detect no appreciable difference in the Jag1 expression pattern relative to wild type controls ($n=3/3$ mutants; data not shown). At older ages (P0–P3), Jag1 expression is progressively diminished with increasing removal of *Rbpj* function (Jia et al., 2007; Rowan et al., 2008). But because multiple transition zone and AEL markers and lens size and morphology are all affected in the postnatal lens, these experiments all suggest that *Jag1* expression is not regulated by *Notch-Rbpj* feedback in the embryonic lens.

Discussion

Here we demonstrate that elimination of *Jag1* from early stages of lens development has a moderate effect on primary fiber cell formation, but it is catastrophic for lens cell growth and secondary fiber cell genesis. In *Jag1* conditional mutants, AEL progenitor cells and newly postmitotic equatorial transition zone cells cease developing prenatally. This leads to loss of the AEL and transition zone around birth, and in the adult eye, anterior chamber defects that include aphakia.

Jag1 and Rbpj lens mutants differ in phenotypic severity

Removal of the *Notch* pathway effector *Rbpj* during early embryonic lens development results in abnormal progenitor cell growth and differentiation, postnatal lens degeneration and adult microphthalmic eyes, with dysgenic lenses, anterior chamber deformities (Jia et al., 2007; Rowan et al., 2008). Perhaps not surprisingly these phenotypes are largely identical to those of *Jag1* lens mutants, except that nearly all *Jag1* phenotypes are more severe. Because *Rbpj*, in *Notch* receiving cells, integrates all canonical pathway input, logically the loss of *Rbpj* should be just as severe, if not more so, than that of a single ligand or receptor. But, signal pathway effectors like the *Rbpj* orthologue *Su*(*H*) can have multiple, opposing functions (Koelzer and Klein, 2003; Koelzer and Klein, 2006; Morel and Schweisguth, 2000). Both vertebrate Rbpj and Su(H) proteins act as transcriptional repressors, unless they are in a complex with Notch^{IC} and MAML/mastermind where they behave as transcriptional activators (reviewed in Lai, 2002). Thus, deletion of *Rbpj* simultaneously removes both repressor and activator activities, thereby modulating the *Notch*-dependent functions. Furthermore, Rbpj can complex with the bHLH factor Ptf1a, independent of *Notch* signaling (Hori et al., 2008; Masui et al., 2007), meaning that not all *Rbpj* functions in the lens may be *Notch*-dependent.

Although we strongly favor the idea that opposing *Rbpj* functions are sufficient to dampen its mutant phenotypes during lens growth and differentiation, there are other potential explanations for the more severe defects in *Jag1* mutants lenses. First, it is plausible that the Le-Cre transgene may delete the *Jag1* floxed allele more efficiently than it does the *Rbpj* floxed allele. Second, the Rbpj protein may perdure longer than Jag1 protein after Cre-mediated deletion. Our data are consistent with this particular idea, since Jag1 protein is completely removed within 24 h of Le-Cre deletion, resulting in total loss of the *Rbpj* target gene *Hes1* two days earlier than in *Rbpj* conditional mutants (Figs 6 and Rowan et al., 2008). Finally, it is plausible that *Jag1* may regulate some aspect of lens development independent of canonical *Notch* signaling (Six et al., 2004). In support of this possibility, we observed that Ecadherin and Foxe3 expression are much more severely affected in *Jag1* conditional mutants. Further experiments are needed to distinguish among these possibilities. It will be critical to determine the number of *Notch* ligands, receptors and *Rbpj* downstream target genes that are present in the embryonic lens, and whether each one acts in the transition zone, fiber cell-AEL boundary, or both.

Does Jag1 transduce lateral inductive or lateral inhibition signals in the lens?

Activation of *Notch* signaling can either prevent (lateral inhibition) or promote (lateral induction) ligand production (reviewed in Eddison et al., 2000; Lewis, 1998). In the first situation, a ligand-producing cell successfully signals its neighbor to reduce ligand expression, which reinforces the ability of the first cell to maintain or enhance its own ligand production. Therefore, cells with differing amounts of ligand adopt discrete developmental fates. In tissues where lateral inhibition is active, ligand expression is predicted to be mosaic, in either an on-off or high-low configuration. Two well-known examples of lateral inhibition occur during *C. elegans* vulval and *Drosophila* sensory bristle formation (Simpson, 1990; Sternberg, 1988). Alternatively, lateral inductive signaling occurs when a ligand-expressing cell stimulates those nearby to turn up ligand expression, promoting coordinated cell fate specification among a group of cells. Here, ligand expression is predicted to be patchy with precise boundaries. Lateral inductive signaling has been well studied in the *Drosophila* wing margin, vertebrate limb bud and inner ear (reviewed in Irvine and Vogt, 1997; Lewis, 1998).

Across embryonic lens development, the *Jag1* expression pattern changes several times, but is never mosaic, as would be expected for lateral inhibition. In the lens placode and vesicle, Jag1 protein and mRNA expression are essentially ubiquitous, with progressive restriction to the posterior vesicle during primary fiber cell formation. Here, posterior vesicle cells also display strong, uniform Jag1 expression. Another hallmark of lateral inhibition is temporal acceleration of differentiation, which we did not observe in *Jag1* lens mutants for primary or secondary fiber genesis. Although the proportions of primary fiber cell differentiation and proliferation shift, neither are accelerated, or abolished, by the loss of *Jag1*. Therefore, a different *Notch* ligand probably regulates lateral inhibition for at least the first wave of fiber differentiation.

When secondary fiber cell production initiates, Jag1 expression is further restricted to the transition zone. This particular domain has a sharp anterior boundary with the AEL, but paradoxically is graded peripheral to central. Jag1+ cells passing out of the transition zone appear to cooperatively adopt a secondary fiber cell fate, and without *Jag1* this larger, second wave of fiber cell formation ceases by birth. Intriguingly, the transition zone domain is reminiscent of Jag1 expression in prosensory patches of the inner ear (Brooker et al., 2006; Kiernan et al., 2005; Kiernan et al., 2006). Based on both its expression pattern and genetic requirements in the lens, we propose that *Jag1* transduces a lateral inductive signal during primary fiber cell genesis that is relatively weak, perhaps because a separate lateral inhibitory signal is the primary mode of *Notch* regulation for this cell type. Then during secondary fiber cell formation, *Jag1*-dependent inductive signaling becomes concentrated in the transition zone. Because loss of *Jag1* causes increased number of fiber cell differentiating at the same time that progenitor cell proliferation decreases, it remains unclear from our analyses whether at the equator *Jag1* strictly regulates cell cycle exit.

Finally, Jag1 is expressed along the anterior edge of fiber cells, where they border the AEL growth zone. AEL progenitor cells are devoid of Jag1 ligand, but express at least two *Notch* receptors, plus the downstream effectors *Rbpj* and *Hes1* (Bao and Cepko, 1997; Jia et al., 2007; Rowan et al., 2008; Weinmaster et al., 1992). Thus, a lateral inhibitory signal between fiber cells and the AEL might be predicted here, in which activated Notch in AEL cells suppresses Jag1 expression. However, loss of *Rbpj* or *Hes1* did not result in derepression of Jag1 in the AEL; nor did misexpression of activated Notch downregulate Jag1 (Rowan et al., 2008, this paper). This strongly implies that if a *Notch* lateral inhibition signal does traverse this tissue boundary, *Jag1* neither communicates it, nor is regulated by such a signal. Instead, our findings are consistent with the idea that Jag1+ fiber cells act concertedly to keep Notch activity high in the AEL, which blocks premature progenitor cell differentiation. Interestingly, *Notch* signaling at AEL/fiber cell boundary is quite similar to the dorsal–ventral tissue boundaries of the fly wing and vertebrate limb bud, where *Serrate*-expressing cells abut the wing margin or limb AER, which each undergo outgrowth (Irvine, 1999; Irvine and Vogt, 1997). In both types of appendages, the Serrate-mediated signal is modulated to be unidirectional through the activity of *fringe* (Irvine and Wieschaus, 1994). It is tantalizing to speculate whether a mammalian *fringe-like* gene is present the developing lens. In the future it will be important to test for expression and function of other *Notch* pathway ligands at the AEL-fiber cell boundary, and remove *Jag1* function specifically in fiber cells. The latter experiment should indicate if *Jag*1 transduces a lateral inductive signal to anterior proliferating lens progenitor cells, and help assign particular *Jag1* phenotypes to fiber cell or transition zone expression domains.

Acknowledgments

The authors thank Julian Lewis for *Jag1*^{CKO} mutant mice; Tasuku Honjo for *Rbpj*^{CKO} mice; Ruth Ashery-Padan and Richard Lang for Le-

Cre transgenic mice; Peter Carlsson, Marten Smidt, Richard Lang, Grant Mastick and Guillermo Oliver for antibody reagents; Sheldon Rowan, Senthil Saravanamuthu, Peggy Zelenka and Brian Gebelein for valuable discussion and critical reading of this manuscript. This work was supported by NIH grants EY18097 and CHRF research funds to NLB.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2009.01.015.

References

Ashery-Padan, R., Marquardt, T., Zhou, X., Gruss, P., 2000. Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. Genes Dev. 14, 2701–2711.
Bao, Z.Z., Cepko, C.L., 1997. The expression and function of Notch pathway genes in the developing rat eye. J. Neurosci. 17, 1425–1434.
Beebe, D., Garcia, C., Wang, X., Rajagopal, R., Feldmeier, M., Kim, J.Y., Chytil, A., Moses, H., Ashery-Padan, R., Rauchman, M., 2004. Contributions by members of the TGFbeta superfamily to lens development. Int. J. Dev. Biol. 48, 845–856.
Blixt, A., Mahlapuu, M., Aitola, M., Pelto-Huikko, M., Enerback, S., Carlsson, P., 2000. A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. Genes Dev. 14, 245–254.
Blixt, A., Landgren, H., Johansson, B.R., Carlsson, P., 2007. Foxe3 is required for morphogenesis and differentiation of the anterior segment of the eye and is sensitive to Pax6 gene dosage. Dev. Biol. 302, 218–229.
Brooker, R., Hozumi, K., Lewis, J., 2006. Notch ligands with contrasting functions: Jagged1 and Delta1 in the mouse inner ear. Development 133, 1277–1286.
Brown, N.L., Kanekar, S., Vetter, M.L., Tucker, P.K., Gemza, D.L., Glaser, T., 1998. Math5 encodes a murine basic helix–loop–helix transcription factor expressed during early stages of retinal neurogenesis. Development 125, 4821–4833.
Cabrera, C.V., 1990. Lateral inhibition and cell fate during neurogenesis in *Drosophila*: the interactions between scute, Notch and Delta. Development 110, 733–742.
Cain, S., Martinez, G., Kokkinos, M.I., Turner, K., Richardson, R.J., Abud, H.E., Huelsken, J., Robinson, M.L., de Iongh, R.U., 2008. Differential requirement for beta-catenin in epithelial and fiber cells during lens development. Dev. Biol.
Cvekl, A., Duncan, M.K., 2007. Genetic and epigenetic mechanisms of gene regulation during lens development. Prog. Retin. Eye Res. 26, 555–597.
Davis, R., Turner, D.L., 2001. Vertebrate hairy and Enhancer of split related proteins: transcriptional repressor regulating celluar differentiation and embryonic patterning. Oncogene 20, 8342–8357.
Eddison, M., Le Roux, I., Lewis, J., 2000. Notch signaling in the development of the inner ear: lessons from *Drosophila*. Proc. Natl. Acad. Sci. U. S. A. 97, 11692–11699.
Faber, S.C., Robinson, M.L., Makarenkova, H.P., Lang, R.A., 2002. Bmp signaling is required for development of primary lens fiber cells. Development 129, 3727–3737.
Glaser, T., Jepeal, L., Edwards, J.G., Young, S.R., Favor, J., Maas, R.L., 1994. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat. Genet. 7, 463–471.
Graw, J., 2003. The genetic and molecular basis of congenital eye defects. Nat. Rev., Genet. 4, 876–888.
Gridley, T., 2003. Notch signaling and inherited disease syndromes. Hum. Mol. Genet. 12 (Spec No 1), R9–13.
Grimm, C., Chatterjee, B., Favor, J., Immervoll, T., Löster, J., Klopp, N., Sandulache, R., Graw, J., 1998. Aphakia (ak), a mouse mutation affecting early eye development: fine mapping, consideration of candidate genes and altered Pax6 and Six3 gene expression pattern. Dev. Genet. 23, 299–316.
Han, H., Tanigaki, K., Yamamoto, N., Kuroda, K., Yoshimoto, M., Nakahata, T., Ikuta, K., Honjo, T., 2002. Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. Int. Immunol. 14, 637–645.
Hori, K., Cholewa-Waclaw, J., Nakada, Y., Glasgow, S.M., Masui, T., Henke, R.M., Wildner, H., Martarelli, B., Beres, T.M., Epstein, J.A., Magnuson, M.A., Macdonald, R.J., Birchmeier, C., Johnson, J.E., 2008. A nonclassical bHLH Rbpj transcription factor complex is required for specification of GABAergic neurons independent of Notch signaling. Genes Dev. 22, 166–178.
Ilagan, M.X., Kopan, R., 2007. SnapShot: notch signaling pathway. Cell 128, 1246.
Irvine, K.D., 1999. Fringe, Notch, and making developmental boundaries. Curr. Opin. Genet. Dev. 9, 434–441.
Irvine, K.D., Wieschaus, E., 1994. fringe, a Boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. Cell 79, 595–606.
Irvine, K.D., Vogt, T.F., 1997. Dorsal–ventral signaling in limb development. Curr. Opin. Cell Biol. 9, 867–876.
Jia, J., Lin, M., Zhang, L., York, J.P., Zhang, P., 2007. The notch signaling pathway controls the size of the ocular lens by directly suppressing p57Kip2 expression. Mol. Cell. Biol. 27, 7236–7247.
Kageyama, R., Ohtsuka, T., Kobayashi, T., 2007. The Hes gene family: repressors and oscillators that orchestrate embryogenesis. Development 134, 1243–1251.
Kiernan, A.E., Cordes, R., Kopan, R., Gossler, A., Gridley, T., 2005. The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. Development 132, 4353–4362.
Kiernan, A.E., Xu, J., Gridley, T., 2006. The notch ligand JAG1 is required for sensory progenitor development in the mammalian inner ear. PLoS Genet. 2, e4.
Kim, J.I., Li, T., Ho, I.C., Grusby, M.J., Glimcher, L.H., 1999. Requirement for the c-Maf transcription factor in crystallin gene regulation and lens development. Proc. Natl. Acad. Sci. U. S. A. 96, 3781–3785.
Koelzer, S., Klein, T., 2003. A Notch-independent function of Suppressor of Hairless during the development of the bristle sensory organ precursor cell of *Drosophila*. Development 130, 1973–1988.
Koelzer, S., Klein, T., 2006. Regulation of expression of Vg and establishment of the dorsoventral compartment boundary in the wing imaginal disc by Suppressor of Hairless. Dev. Biol. 289, 77–90.
Kopan, R., 2002. Notch: a membrane-bound transcription factor. J. Cell. Sci. 115, 1095–1097.
Lai, E.C., 2002. Keeping a good pathway down: transcriptional repression of Notch pathway target genes by CSL proteins. EMBO Rep. 3, 840–845.
Lang, R.A., 2004. Pathways regulating lens induction in the mouse. Int. J. Dev. Biol. 48, 783–791.
Lee, H.Y., Wroblewski, E., Philips, G.T., Stair, C.N., Conley, K., Reedy, M., Mastick, G.S., Brown, N.L., 2005. Multiple requirements for Hes 1 during early eye formation. Dev. Biol. 284, 464–478.
Lewis, J., 1998. Notch signalling and the control of cell fate choices in vertebrates. Semin. Cell Dev. Biol. 9, 583–589.
Louvi, A., Artavanis-Tsakonas, S., 2006. Notch signalling in vertebrate neural development. Nat. Rev., Neurosci. 7, 93–102.
Lovicu, F.J., McAvoy, J.W., 2001. FGF-induced lens cell proliferation and differentiation is dependent on MAPK (ERK1/2) signalling. Development 128, 5075–5084.
Mastick, G.S., Andrews, G.L., 2001. Pax6 regulates the identity of embryonic diencephalic neurons. Mol. Cell. Neurosci. 17, 190–207.
Masui, T., Long, Q., Beres, T.M., Magnuson, M.A., MacDonald, R.J., 2007. Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. Genes Dev. 21, 2629–2643.
McAvoy, J.W., Chamberlain, C.G., de Iongh, R.U., Hales, A.M., Lovicu, F.J., 1999. Lens development. Eye 13 (Pt 3b), 425–437.
Medina-Martinez, O., Brownell, I., Amaya-Manzanares, F., Hu, Q., Behringer, R.R., Jamrich, M., 2005. Severe defects in proliferation and differentiation of lens cells in Foxe3 null mice. Mol. Cell. Biol. 25, 8854–8863.
Morel, V., Schweisguth, F., 2000. Repression by suppressor of hairless and activation by Notch are required to define a single row of single-minded expressing cells in the *Drosophila* embryo. Genes Dev. 14, 377–388.
Ogino, H., Fisher, M., Grainger, R.M., 2008. Convergence of a head-field selector Otx2 and Notch signaling: a mechanism for lens specification. Development 135, 249–258.
Rieger, D.K., Reichenberger, E., McLean, W., Sidow, A., Olsen, B.R., 2001. A double-deletion mutation in the Pitx3 gene causes arrested lens development in aphakia mice. Genomics 72, 61–72.
Robinson, M.L., 2006. An essential role for FGF receptor signaling in lens development. Semin Cell. Dev. Biol. 17, 726–740.
Rowan, S., Conley, K.W., Le, T.T., Donner, A.L., Maas, R.L., Brown, N.L., 2008. Notch signaling regulates growth and differentiation in the mammalian lens. Dev. Biol. 321, 111–122.
Semina, E.V., Reiter, R.S., Murray, J.C., 1997. Isolation of a new homeobox gene belonging to the Pitx/Rieg family: expression during lens development and mapping to the aphakia region on mouse chromosome 19. Hum. Mol. Genet. 6, 2109–2116.
Semina, E.V., Ferrell, R.E., Mintz-Hittner, H.A., Bitoun, P., Alward, W.L., Reiter, R.S., Funkhauser, C., Daack-Hirsch, S., Murray, J.C., 1998. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. Nat. Genet. 19, 167–170.
Simpson, P., 1990. Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*. Development 109, 509–519.
Six, E.M., Ndiaye, D., Sauer, G., Laabi, Y., Athman, R., Cumano, A., Brou, C., Israel, A., Logeat, F., 2004. The notch ligand Delta1 recruits Dlg1 at cell–cell contacts and regulates cell migration. J. Biol. Chem. 279, 55818–55826.
Song, N., Schwab, K.R., Patterson, L.T., Yamaguchi, T., Lin, X., Potter, S.S., Lang, R.A., 2007. pygopus 2 has a crucial, Wnt pathway-independent function in lens induction. Development 134, 1873–1885.
Sternberg, P.W., 1988. Lateral inhibition during vulval induction in *Caenorhabditis elegans*. Nature 335, 551–554.
Valleix, S., Niel, F., Nedelec, B., Algros, M.P., Schwartz, C., Delbosc, B., Delpech, M., Kantelip, B., 2006. Homozygous nonsense mutation in the FOXE3 gene as a cause of congenital primary aphakia in humans. Am. J. Hum. Genet. 79, 358–364.
Weinmaster, G., Roberts, V.J., Lemke, G., 1992. Notch2: a second mammalian Notch gene. Development 116, 931–941.
Xu, L., Overbeek, P.A., Reneker, L.W., 2002. Systematic analysis of E-, N- and P-cadherin expression in mouse eye development. Exp. Eye Res. 74, 753–760.
Xue, Y., Gao, X., Lindsell, C.E., Norton, C.R., Chang, B., Hicks, C., Gendron-Maguire, M., Rand, E.B., Weinmaster, G., Gridley, T., 1999. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. Hum. Mol. Genet. 8, 723–730.
Zhao, H., Yang, T., Madakashira, B.P., Thiels, C.A., Bechtle, C.A., Garcia, C.M., Zhang, H., Yu, K., Ornitz, D.M., Beebe, D.C., Robinson, M.L., 2008. Fibroblast growth factor receptor signaling is essential for lens fiber cell differentiation. Dev. Biol. 318, 276–288.